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Ewa Brzezińska, Monika Kozłowska

Effect of sunlight on phenolic compounds accumulation in coniferous plants

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Abstract: The effect of light conditions on the accumulation of phenolic compounds was investigated in the needles of one-year and two-year old shoots, collected from perennial specimens of common yew (*Taxus baccata* L.), American arborvitae (*Thuja occidentalis* L.) and common juniper (*Juniperus communis* L.), growing in an urban garden. The content of total phenols and flavones was partially related to the light requirement or light tolerance of conifer tree. In *Taxus* needles, a shade-loving species, higher accumulation of phenolic metabolites was observed under shaded condition (in two-year old shoots) and was linked with the growth intensity in spring. In *Thuja* and particularly in *Juniperus,* the level of phenolics was related to the insolation intensity, probably so as a part of the adaptation mechanism.

Additional key words: light conditions, *Taxus baccata, Thuja occidentalis, Juniperus communis,* flavones

Address: Department of Plant Physiology, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland, e-mail:monkozlo@jay.au.poznan.pl

Introduction

Phenolic compounds are a widely distributed class of secondary metabolites, located in vacuoles of plant cells, in the intercellular space, as well as on plant surface. They form a numerous group of compounds with a diverse chemical structure, and include simple phenols, such as phenolic acids, flavonoids (flavones, flavonols and anthocyanines), and polymerized phenols, such as tannins and lignins.

These compounds are characterized by widespread distribution and various functions (Harborne 1993). They participate in defense responses to pathogens and in allelopathy, protect cell structures against adverse action of excess photochemical energy and ultraviolet radiation, especially UV-B. Phenolics found in flowers and fruit give colour to those organs (Winkel-Shirley 2001) and attract animals carrying pollen and dispersing seeds. Flavonoids also participate in regulation of auxin biosynthesis and are one of the morphogenetic photoreceptors, referred to as cryptochrome. They may also be responsible for the taste and firmness of plant tissues (Horbowicz 2000).

One of the environmental factors significantly affecting the accumulation of these metabolites is sunlight (Karolewski et al. 2007). Under strong insolation the intensity of photosynthetically active radiation is enhanced (Kubaćkova et al. 1994), but the same is also true of ultraviolet radiation, especially UV-B, under the influence of which a stimulation of phenolic compounds biosynthesis was observed (Dixon and Paiva 1995). In plants subjected to strong radiation, enhanced accumulation of phenolic compounds was correlated with expression and activation of key enzymes of phenylpropanoid pathway, i.e. phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), participating in flavonoid biosynthesis (Schnitzler et al. 1997, Sarma and Sharma 1999, Hollósy 2002).

Apart from forest areas, coniferous plants are widely used in gardens and parks. Their phenotypic characters may depend on locality and can be modified by adverse environmental conditions. Phytochemical properties of conifers and their acclimation to irradiance conditions play an important role in resistance to environmental stress (Harborne 1993).

In the present study, it was hypothesized that sunlight conditions modify the accumulation of phenolic compounds of ornamental conifers growing in an urban park, taking into consideration the development phase of plants. The shade-loving *Taxus baccata* and the light-demanding *Juniperus communis* were investigated in comparison to the light and shade tolerant *Thuja occidentalis*.

Material and methods

Plant material consisted of several-year old specimens of common yew (*Taxus baccata* L.), American arborvitae (*Thuja occidentalis* L.) and common juniper (*Juniperus communis* L.), growing in the Dendrological Garden of Poznań (52°25'N, 16°56'E) on two localities: under full solar irradiance and under shade. The reduction of solar irradiance, generated by surrounding trees, was two-fold for *Taxus* and *Juniperus* and even four-fold for *Thuja*, measured under sun days. Material was collected from perennial specimens of trees or shrubs, on three dates in 2–3-week intervals in the period of May until June. For *Taxus* and *Thuja* three trees/shrubs and for *Juniperus* two shrubs were chosen for both light conditions, and analyzed in three repetitions. Indicator parts of plants were needles of one- and two-year old shoots.

For phenolic compound determination frozen comminuted needles (200 mg) were flooded with 80% methanol (5 ml) and left to stand for 24 h. After that needles were homogenized and additionally washed with 2 ml of methanol. Combined liquids were centrifuged at 16 000g and the supernatants were evaporated in a speed vacuum concentrator (Heto Lab Equipment A/S, Denmark) to give aqueous phase. This phase was double extracted with 2 × 5 ml of ethyl acetate (15 min on a Vortex shaker). Combined ethyl fractions were evaporated to dryness. Residues were dissolved in 1 ml 80% methanol. Total phenolics were measured by Folin-Ciocalten's reagent (Sigma) according to Swain and Hillis (1959) and expressed as p-coumaric acid equivalent.

Flavones were assayed according to Caldwell et al. (1994) by measuring absorbance of methanol extracts in a UV/VIS spectrophotometer at a wavelength of 305 nm. Relative contents of these compounds were expressed in absorbance units corresponding to dry weight of leaves (DW).

Experimental data were subjected to Anova statistical analysis and the Tukey's HSD test using the Statistica 6 software.

Results

The highest constitutive level of total phenolic compounds was found for *Taxus*, while *Thuja* and *Juniperus* contained two, and even several times lower content of these metabolites (Fig. 1). Content of phenolics, irrespective of the sunlight position, was markedly dominant in two-year old shoots. The exception were young *Taxus* needles which contained a higher level of phenolics. Under the sunny conditions, needles of one-year old *Taxus* shoots contained a higher amount of these compounds, while in two-year olds it was approx. 20% lower.

In *Thuja* and *Juniperus* needles growing under shaded conditions the content of phenolic compounds was significantly lower as compared to full solar irradiance (Table 1). This relation pertained to both young and older shoots.

Figure 2 presents data concerning the flavones content. In general, the level of these pigments was of similar tendency as with total phenolics, so the highest in *Taxus* and lower under shaded conditions (referring to all genotypes). The content of flavones was significantly reduced in two-year old *Taxus* needles when compared to the young ones (sunny position), in contrast to *Thuja* and *Juniperus* where the accumulation increases with age (Table 2).

The course of accumulation was inconsiderable related to the growth intensity in spring. Among the in-

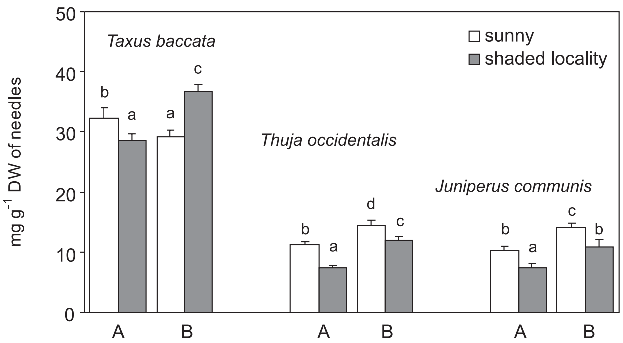

Fig. 1. Content of total phenolic compounds in common yew (*Taxus baccata*), American arborvitae (*Thuja occidentalis*) and juniper (*Juniperus communis*) needles as dependent on light conditions; A – young needles, B – two-year old needles (mean of three dates done in May-June period)

Table 1. Summary of ANOVA results for phenolic compounds by light and age treatment.

Source of variation	*Taxus*			*Thuja*			Juniperus		
	d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*
Light (L)	1	2,93	NS	1	14,22	< 0,01	1	37,21	< 0,01
Age (A)	1	2,11	NS	1	21,01	< 0,01	1	51,07	< 0,01
L × A	1	15,33	< 0,01	1	0,44	NS	1	0,14	NS
Error	68			32			56		

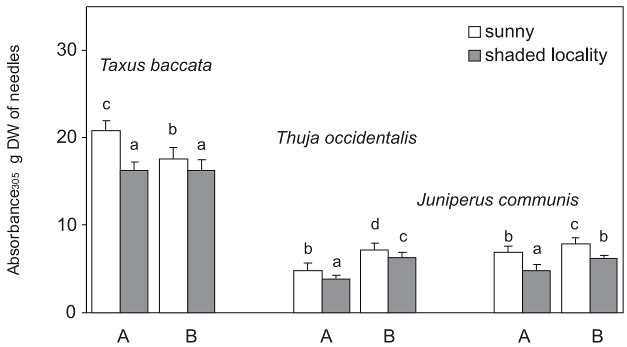

Fig. 2. Content of flavones in common yew (*Taxus baccata*), American arborvitae (*Thuja occidentalis*) and juniper (*Juniperus communis*) needles as dependent on light conditions; A – young needles, B – two years old needles (mean of three dates done in May-June period)

Table 2. Summary of ANOVA results for flavones by light and age treatment

Source of variation	*Taxus*			*Thuja*			*Juniperus*		
	d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*
Light (L)	1	14,23	< 0,01	1	17,18	< 0,01	1	41,48	< 0,01
Age (A)	1	4,18	NS	1	124,64	< 0,01	1	11,14	< 0,01
L × A	1	4,13	NS	1	0,003	NS	1	0,54	NS
Error	44			44			68		

vestigated conifers, only in *Taxus* did the content of phenolics change from the May determination to the end of June (Fig. 3).

Due to considerably lower level of dry matter in young needles (Table 3), the amount of phenolic compounds in fresh leaf tissues in no case exceed 40–50% of the content in elder needles.

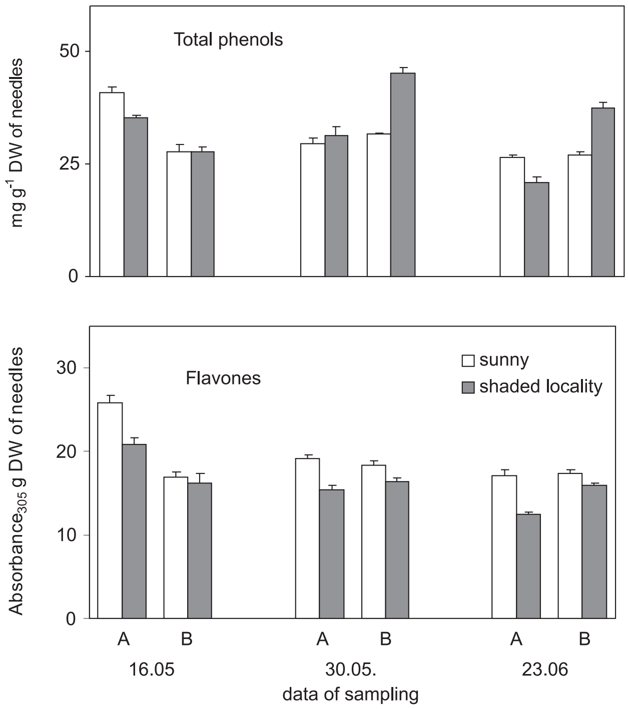

Fig. 3. Comparison of total phenols and flavones content in common yew (*Taxus baccata*) during the spring growth

Table 3. Mean content of dry weight (%) measured in May till half of Juny

Light conditions	*Taxus*		*Thuja*		*Juniperus*	
	1*	2*	1*	2*	1*	2*
Sunny	32,30	45,21	28,14	49,08	30,83	44,86
Shaded	28,13	43,11	30,18	46,75	28,11	41,40

* 1 – one-year old, and 2 – two-year old needles

Discussion

Species from genera *Taxus*, *Thuja* and *Juniperus* vary in terms of their structure and habitat requirements, especially including sunlight conditions, which may have a considerable effect on their response to radiation stress, and thus on the accumulation of phenolic compounds. *Taxus baccata* is a shade-loving tree but *Juniperus communis* a light-demanding one, thus they are in the extreme of light requirements. Nevertheless, both in natural ecosystems as well as in parks and gardens, the environmental conditions including irradiation are changeable. This is possible due to the mechanisms of adaptation and/or of acclimation to the excess or deficiency of photochemical energy. At the same time radiation may modify secondary metabolism also involved in acclimation, protecting ahead the photoinhibitory processes. Our results confirm that light played a significant role in the accumulation of phenolic compounds, and to a different degree in conifers. Its effect was considerable in *Juniperus*, where the accumulation of phenolic compounds, including flavones most of all, was related with the growing position; it was 20–30% higher in a sunny than in a shaded position. It is a plant with high light requirements, although in terms of other factors, e.g. soil conditions, its needs are relatively small. These observations confirm the general opinion on the effect of light on synthesis of phenolic compounds (Siegelman 1964, Kubaćkova et al. 1994).

On the contrary, the content of total phenolics in *Taxus* needles, a shade-loving tree, was higher in a shaded than in a sunny locality. While the activity of phenylpropanoid enzymes is light-dependent (e.g. phenylalanine ammonia-lyase and chalcone synthase), the accumulation of phenolics is probably due to biochemical adaptation to the shade condition. Thus, a high level of secondary metabolites in *Taxus* and, moreover, a higher level in elder leaves (up to two-year olds) are important properties of this tree. Similar trends were also supported for Scots pine (Giertych 2001) and for rice (Kar and Mishra 1976) – not shade-adapted plants, where the insufficient illumination stimulated the synthesis of phenolic metabolites.

The phenolic compounds content in *Thuja*, a light tolerant genera, was similar to *Juniperus*, but the accumulation of flavones was significantly higher in the elder scales. These observations are consistent with the results reported by Robakowski and Laitat (1999), as well as Yakimchuk and Hoddinott (1994), who found increased flavonoid content, which was accompanied by enhanced intensity of UV-B radiation. In the response of *Thuja* to UV-B, the highest changes referred to the appearance of two identified free flavonoids (Kozłowska et al. 2007).

Another aspect of the investigation was the effect of age and leaf maturity on the accumulation of phenolic compounds. The concentration of these metabolites most of all was influenced by the ontogenetic leaf age (Laitinen et al., 2000; Kozłowska et al., 2005; Andreotti et al. 2006). Karolewski and Giertych (1995), Sullivan et al. (1996) and DeLucia et al. (1992) showed that the level of phenolics increased along with the needles age. This seems justified, since secondary products may be formed and accumulated only as a result of an "excess" of primary metabolites. Thus, it was expected that due to the needle several years lifetime, the accumulation of phenolics can be cumulative. In fact, contents of these metabolites in two-year old shoots were higher in arborvitae and juniper but not in common yew. Probably, in common yew needles part of these compounds are involve in formation of phenolic bound forms. In Scots pine the increase of phenolics pertained only to needles from the first year of growth, while in the next three years content of these metabolites was relatively stable (Esterbauer and Grill (1975).

Summing up, it was confirmed that light conditions are a significant factor affecting the accumulation of phenolic compounds, responsible for the adaptation to changeable environment. The content of these metabolites was dependent on the plant species and the age of the plant. Among analyzed genera, the highest constitutive level was recorded in *Taxus*, and, moreover, the accumulation of total phenols was higher under shaded condition.

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